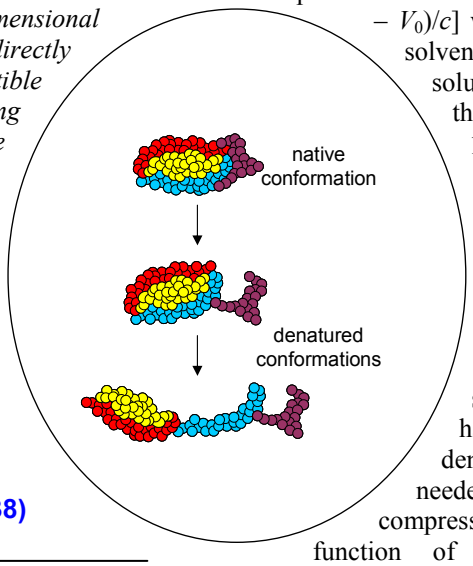
Protein Stability via Speed of Sound and Density Measurements

The stability of proteins is a key concern for a variety of reasons. Proteins consist of chains of amino acids that are normally folded into a particular three-dimensional conformation. The shape of a protein is directly linked to its function. Proteins are susceptible to losing their native conformation (becoming "denatured"), which causes them to lose their biological activity. NIST researchers studied the effect of changes in pressure on conformational stability of a globular protein, bovine serum albumin. This protein was selected because of the availability of SRM 927 Bovine Serum Albumin, which is necessarily well characterized, and its stability against denaturation has been studied in detail.



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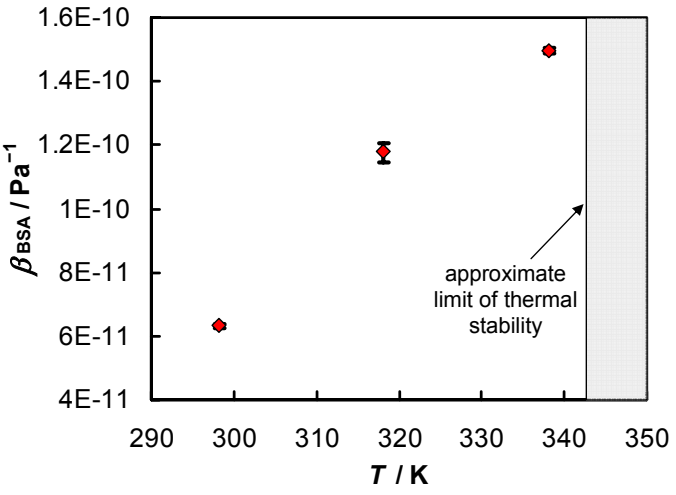
In an aqueous environment, protein folding and denaturation is driven by changes in the chemical potential of water. The chemical potential of water depends on the temperature, the concentration of ionic compounds, the pH, and the pressure. It is the latter dependence that motivates this research. Proteins are the most pressure-sensitive class of biomolecules. Increasing the pressure decreases the stability of some proteins and increases the stability of other proteins. Consequently, some proteins have an increased shelf life at increased pressure, which is of potential use for protein-based pharmaceuticals, while other proteins are rapidly denatured at high pressure, which is of potential use for low-temperature sterilization, food preservation, and vaccine production. The pressure sensitivity of proteins leads to the hypothesis that the adiabatic compressibility of proteins in aqueous solution, $\beta_{protein}$, can be used to measure the conformational stability of biologically active proteins. It is reasonable to believe that conformational stability and compressibility are related because both depend, in part, on the conformational flexibility and internal solvation of the protein.

To test this hypothesis we need to determine how $\beta_{protein}$ changes when the protein becomes unstable. This can be accomplished for proteins in aqueous solution by measuring the speed of sound and density as functions of concentration and temperature. At each temperature, the partial specific volume ($v°$) of the protein is calculated from the equation $v° = \lim_{c\to 0}\{1 - [(\rho - c)/\rho_0]/c\}$ where ρ is the density of the solution, ρ_0 is the density of water, and c is the protein concentration. Then $\beta_{protein}$ is calculated at each temperature from the equation $\beta_{protein} = (\beta_0/v°)\cdot\lim_{c\to 0}[(\beta/\beta_0 - V_0)/c]$ where β_0 is the compressibility of the solvent, β is the compressibility of the solution, and V_0 is the volume fraction of the protein. The Laplace equation, $\beta = 1/(\rho u^2)$, is used to calculate β and β_0. Low protein concentrations, in the range of (0.1 to 1.0) % mass fraction, ensure the linearity of the extrapolations used to obtain $v°$ and $\beta_{protein}$.

Bovine serum albumin (BSA), a medium-sized globular protein was selected for this work since its stability has been well studied. High-precision density and speed-of-sound data are needed to distinguish changes in protein compressibility. Such data were collected as a function of concentration at three different temperatures using a commercial density and speed-of-sound analyzer. At each temperature, the concentration dependence of density and speed of sound were used to calculate the adiabatic compressibility of BSA, β_{BSA}. In the figure, β_{BSA} is plotted as a function of temperature with error bars indicating the precision of four replicate measurements. The figure demonstrates that the data produced by this instrument are sufficiently precise to observe changes in β_{BSA} as the solution temperature changes from (298.15 to 338.15) K. This is a proof-of-concept result for our experimental design.



During the course of this work two important technical challenges have come to light, which, if overcome, will greatly facilitate future work in this area. First, the concentrations of the protein solutions must be determined more accurately. To date, we have prepared BSA solutions gravimetrically from freeze-dried commercial powders of BSA, which contain significant amounts of water. By using either Karl Fischer titration or mass loss on oven drying, freeze-dried BSA with a claimed purity of 99+ % was found to contain (5 to 10) % water mass fraction. Unfortunately, there is a systematic difference between water determinations with these two methods, which prevents a confident correction for the water content, and leads to a significant uncertainty in the BSA concentration. To circumvent this problem we plan to use the optical absorbance of BSA to directly relate the concentration of our solutions with the concentration of SRM 927 (7 % BSA in aqueous solution).

Additionally, the conformational purity of the protein must be established. We intend to use hydrogen exchange mass spectrometry to determine the fraction of BSA in our samples that is in its native conformation at the beginning and end of our experiments. This will allow us to check the quality of commercially prepared BSA (including SRM 927) with respect to denaturation. It will also help us relate observed changes in compressibility to protein denaturation. We have begun collaborating with Katheryn Resing at the University of Colorado to perform these experiments.

An important goal of this work is to obtain reference-quality data for the partial specific volume of BSA in aqueous solution, which is determined from density measurements at known BSA concentrations, as described above. This would constitute a unique contribution by itself, and would be useful as a reference point for the modeling of protein solvation. We will also collect reference quality data for the extinction coefficient of BSA in aqueous solution, which is useful for concentration determinations.

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